(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 779 929 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
11.04.2001 Bulletin 2001/15

(21) Application number: **95931969.0**

(22) Date of filing: **01.09.1995**

(51) Int Cl.[7]: **C12N 15/867**, C12N 7/00, C12N 15/11, A61K 48/00

(86) International application number:
PCT/EP95/03445

(87) International publication number:
WO 96/07748 (14.03.1996 Gazette 1996/12)

(54) NON SELF-INACTIVATING, EXPRESSION TARGETED RETROVIRAL VECTORS

NICHT SELBSTINAKTIVIERENDE RETROVIRALE VEKTOREN MIT GEZIELTER EXPRESSION

VECTEURS RETROVIRAUX NON AUTO-INACTIVANTS, UTILES POUR UNE EXPRESSION CIBLEE

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
LT LV SI

(30) Priority: **02.09.1994 DK 101794**

(43) Date of publication of application:
25.06.1997 Bulletin 1997/26

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Description

[0001] The present invention relates to retroviral vectors including a vector which undergoes promoter conversion (ProCon vector). The vector system is useful as a gene transfer vehicle for targeted gene therapy.

Background of the Invention

[0002] The use of retroviral vectors for gene therapy has received much attention and currently is the method of choice for the transferral of therapeutic genes in a variety of approved protocols both in the USA and in Europe (Kotani et al., 1994). However most of these protocols require that the infection of target cells with the retroviral vector carrying the therapeutic gene occurs in vitro, and successfully infected cells are then returned to the affected individual (Rosenberg et al., 1992; for a review see Anderson, 1992). Such ex vivo gene therapy protocols are ideal for correction of medical conditions in which the target cell population can be easily isolated (e.g. lymphocytes). Additionally the ex vivo infection of target cells allows the administration of large quantities of concentrated virus which can be rigorously safety tested before use.

[0003] Unfortunately, only a fraction of the possible applications for gene therapy involve target cells that can be easily isolated, cultured and then reintroduced. Additionally, the complex technology and associated high costs of ex vivo gene therapy effectively preclude its disseminated use world-wide. Future facile and cost-effective gene therapy will require an in vivo approach in which the viral vector, or cells producing the viral vector, are directly administered to the patient in the form of an injection or simple implantation of retroviral vector producing cells.

[0004] This kind of in vivo approach, of course, introduces a variety of new problems. First of all, and above all, safety considerations have to be addressed. Virus will be produced, possibly from an implantation of virus producing cells, and there will be no opportunity to precheck the produced virus. It is important to be aware of the finite risk involved in the use of such systems, as well as trying to produce new systems that minimize this risk.

[0005] Retroviral vector systems consist of two components (Fig. 1):

1) the retroviral vector itself is a modified retrovirus (vector plasmid) in which the genes encoding for the viral proteins have been replaced by therapeutic genes and marker genes to be transferred to the target cell. Since the replacement of the genes encoding for the viral proteins effectively cripples the virus it must be rescued by the second component in the system which provides the missing viral proteins to the modified retrovirus.

The second component is:

2) a cell line that produces large quantities of the viral proteins, however lacks the ability to produce replication competent virus. This cell line is known as the packaging cell line and consists of a cell line transfected with a second plasmid carrying the genes enabling the modified retroviral vector to be packaged.

[0006] To generate the packaged vector, the vector plasmid is transfected into the packaging cell line. Under these conditions the modified retroviral genome including the inserted therapeutic and marker genes is transcribed from the vector plasmid and packaged into the modified retroviral particles (recombinant viral particles). This recombinant virus is then used to infect target cells in which the vector genome and any carried marker or therapeutic genes becomes integrated into the target cell's DNA. A cell infected with such a recombinant viral particle cannot produce new vector virus since no viral proteins are present in these cells. However the DNA of the vector carrying the therapeutic and marker genes is integrated in the cell's DNA and can now be expressed in the infected cell.

[0007] The essentially random integration of the proviral form of the retroviral genome into the genome of the infected cell (Varmus, 1988) led to the identification of a number of cellular proto-oncogenes by virtue of their insertional activation (Varmus, 1988; van Lohuizen and Berns, 1990). The possibility that a similar mechanism may cause cancers in patients treated with retroviral vectors carrying therapeutic genes intended to treat other pre-existent medical conditions has posed a recurring ethical problem. Most researchers would agree that the probability of a replication defective retroviral vector, such as all those currently used, integrating into or near a cellular gene involving in controlling cell proliferation is vanishingly small. However it is generally also assumed that the explosive expansion of a population of replication competent retrovirus from a single infection event, will eventually provide enough integration events to make such a phenotypic integration a very real possibility.

[0008] Retroviral vector systems are optimized to minimize the chance of replication competent virus being present. However it has been well documented that recombination events between components of the retroviral vector system can lead to the generation of potentially pathogenic replication competent virus and a number of generations of vector systems have been constructed to minimize this risk of recombination (reviewed in Salmons and Günzburg, 1993).

[0009] A further consideration when considering the use of in vivo gene therapy, both from a safety stand point and from a purely practical stand point, is the targeting of retroviral vectors. It is clear that therapeutic genes carried by vectors should not be indiscriminately expressed in all tissues and cells, but rather only in the requisite target cell. This is especially important if the

genes to be transferred are toxin genes aimed at ablating specific tumour cells. Ablation of other, nontarget cells would obviously be very undesirable.

[0010] A number of retroviral vector systems have been previously described that should allow targeting of the carried therapeutic genes (Salmons and Gunzburg, 1993). Most of these approaches involve either limiting the infection event to predefined cell types or using heterologous promoters to direct expression of linked heterologous therapeutic or marker genes to specific cell types. Heterologous promoters are used which should drive expression of linked genes only in the cell type in which this promoter is normally active. These promoters have previously been inserted, in combination with the marker or therapeutic gene, in the body of the retroviral vectors, in place of the *gag*, *pol* or *env* genes.

[0011] The retroviral Long Terminal Repeat (LTR) flanking these genes carries the retroviral promoter, which is generally non-specific in that it can drive expression in many different cell types (Majors, 1990). Promoter interference between the LTR promoter, and heterologous internal promoters, such as the tissue specific promoters described above has been reported. Additionally, it is known that retroviral LTRs harbor strong enhancers that can, either independently, or in conjunction with the retroviral promoter, influence expression of cellular genes near the site of integration of the retrovirus. This mechanism has been shown to contribute to tumorigenicity in animals (van Lohuizen and Bems). These two observations have encouraged the development of Self-Inactivating-Vectors (SIN) in which retroviral promoters are functionally inactivated in the target cell (PCT WO94/29437). Further modifications of these vectors include the insertion of promoter gene cassettes within the LTR region to create double copy vectors (PCT. WO 89/11539). However, in both these vectors the heterologous promoters inserted either in the body of the vector, or in the LTR region are directly linked to the marker/therapeutic gene.

[0012] The previously described SIN vector mentioned above carrying a deleted 3'LTR (PCT WO94/29437) utilize in addition a strong heterologous promoter such as that of Cytomegalovirus (CMV), instead of the retroviral 5'LTR promoter (U3-free 5'LTR) to drive expression of the vector construct in the packaging cell line. A heterologous polyadenylation signal is also included in the 3'LTR (PCT WO94/29437).

[0013] The object of the present invention is the construction of a novel retroviral vector which can be used as a safe gene transfer vehicle for targeted gene therapy with a reduced probability to undergo recombination with the packaging construct. This novel vector carries heterologous promoter and/or regulatory elements in the 3'LTR which, after infection become duplicated and translocated to the 5'LTR in the target cell, eventually controlling expression of marker/therapeutic genes, not directly linked to the promoter, but rather inserted into the body of the vector. This vector does not undergo self-inactivation - but instead promoter exchange, giving rise to the name ProCon for Promoter Conversion.

[0014] Since Promoter Conversion does not result in Self-Inactivation, the retroviral vector will be transcriptionally active in the target cell. However both LTRs will consist to a large extent of heterologous promoter/enhancer sequences in the target cell. This will reduce the likelihood of the integrated vector in the target cell being subject to the same inactivation over long periods as has been described for conventional vectors (Xu ***et al.***, 1989) and also will reduce the chance of recombination with endogenous retroviral sequences to generate potentially pathogenic replication competent virus, increasing the safety of the system.

[0015] In this invention the 5'LTR of the retroviral vector construct is not modified, and expression of the viral vector in the packaging cells is driven by the normal retroviral U3 promoter. Normal retroviral polyadenylation is allowed, and no heterologous polyadenylation signals are included in the 3'LTR. This is important for the development of *in vivo* gene therapy strategies, since the normal physiological regulation of the virus, through the normal viral promoter, and possibly also involving the normal viral control of polyadenylation, will prevail over long periods *in vivo* whilst the packaging cells are producing recombinant virus.

[0016] A further modification of this novel retroviral vector forsees the inclusion of cellular sequences instead of the heterologous promoter and/or regulatory elements. This should allow higher selectivity for site specific recombinantion with cellular sequences to target the integration of retroviral vectors to particular sites in the host cell genome (Saller, 1994).

[0017] To achieve the foregoing and other objects, the invention provides a retroviral vector undergoing promoter conversion comprising a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence, followed by the R and U5 region.

[0018] Said polylinker sequence carries at least one unique restriction site and contains preferably at least one insertion of a heterologous DNA fragment. Said heterologous DNA fragment is preferably selected of regulatory elements and promoters, preferably being target cell specific in their expression, but may also be a DNA fragment with no regulatory function.

[0019] Said heterologous DNA fragment is preferably homologous to one or more cellular sequences. The regulatory elements and promoters are preferably regulatable by transacting molecules.

[0020] Further objects, features and advantages will be apparent from the following description of preferred embodiments of the invention.

[0021] The target cell specific regulatory elements and promoters are selected from one or more elements of the group consisting of Whey Acidic Protein (WAP),

Mouse Mammary Tumour Virus (MMTV), β-lactoglobulin and casein specific regulatory elements and promoters, pancreas specific regulatory elements and promoters including carbonic anhydrase II and β-glucokinase regulatory elements and promoters, lymphocyte specific regulatory elements and promoters including immunoglobulin and MMTV lymphocytic specific regulatory elements and promoters and MMTV specific regulatory elements and promoters conferring responsiveness to glucocorticoid hormones or directing expression to the mammary gland. Said regulatory elements and promoters regulate preferably the expression of at least one of the coding sequences of said retroviral vector. The LTR regions are selected from at least one element of the group consisting of LTRs of Murine Leukaemia Virus (MLV), Mouse Mammary Tumour Virus (MMTV), Murine Sarcoma Virus (MSV), Simian Immunodeficiency Virus (SIV), Human Immunodeficiency Virus (HIV), Human T-cell Leukaemia Virus (HTLV), Feline Immunodeficiency Virus (FIV), Feline Leukaemia Virus (FELV), Bovine Leukaemia Virus (BLV) and Mason-Pfizer-Monkey virus (MPMV).

[0022] The retroviral vector is based preferably either on a BAG vector (Price *et al.*, 1987) or an LXSN vector (Miller and Rosman, 1989).

[0023] The coding sequence is preferably selected from one or more elements of the group consisting of marker genes, therapeutic genes, antiviral genes, antitumour genes, cytokine genes.

[0024] Said marker and therapeutic genes are preferably selected from one or more elements of the group consisting of β-galactosidase gene, neomycin gene, Herpes Simplex Virus thymidine kinase gene, puromycin gene, cytosine deaminase gene, hygromycin gene, secreted alkaline phosphatase gene, guanine phosphoribosyl transferase (gpt) gene, alcohol dehydrogenase gene and hypoxanthine phosphoribosyl transferase (HPRT) gene.

[0025] Another embodiment of the invention envisages the alteration or partial deletion of at least one retroviral sequence required for integration of retroviruses.

[0026] In a further embodiment of the invention a retroviral vector system is provided comprising a retroviral vector as described above as a first component and a packaging cell line harbouring at least one retroviral or recombinant retroviral construct coding for proteins required for said retroviral vector to be packaged.

[0027] The packaging cell line harbours retroviral or recombinant retroviral constructs coding for those retroviral proteins which are not encoded in said retroviral vector. The packaging cell line is preferably selected from an element of the group consisting of psi-2, psi-Crypt, psi-AM, GP+E-86, PA317 and GP+envAM-12, or of any of these supertransfected with recombinant constructs allowing expression of surface proteins from other enveloped viruses.

[0028] Another embodiment of the invention involves the use of a packaging cell line harbouring a recombinant retroviral construct defective in integrase function.

[0029] After introducing the retroviral vector of the invention as described above in a retroviral packaging cell line and infection of a target cell, as described above, a retroviral provirus is provided wherein said polylinker and any sequences inserted in said polylinker in the 3'LTR become duplicated during the process of reverse transcription in the infected target cell and appear in the 5'LTR as well as in the 3'LTR of the resulting provirus.

[0030] The invention includes also mRNA of a retroviral provirus according to the invention and any RNA resulting from a retroviral vector according to the invention.

[0031] A further embodiment of the invention provides non-therapeutical method for introducing homologous and/or heterologous nucleotide sequences into human or animal cells *in vitro* and *in vivo* comprising transfecting a packaging cell line of a retroviral vector system according to the invention with a retroviral vector according to the invention and infecting a target cell population with recombinant retroviruses produced by the packaging cell line. The nucleotide sequences are selected from one or more elements of the group consisting of genes or parts of genes encoding for proteins, regulatory sequences and promoters.

[0032] The retroviral vector, the retroviral vector system and the retroviral provirus as well as RNA thereof is used for producing a pharmaceutical composition for gene therapy in mammals including humans. Furthermore, they are used for targeted integration in homologous cellular sequences.

Promoter conversion

[0033] The present invention uses the principle of promoter conversion typical for retroviruses.

[0034] The retroviral genome consists of an RNA molecule with the structure R-U5-gag-pol-env-U3-R (Fig. 2). During the process of reverse transcription, the U5 region is duplicated and placed at the right hand end of the generated DNA molecule, whilst the U3 region is duplicated and placed at the left hand end of the generated DNA molecule (Fig. 2). The resulting structure U3-R-U5 is called LTR (Long Terminal Repeat) and is thus identical and repeated at both ends of the DNA structure or provirus (Varmus, 1988). The U3 region at the left hand end of the provirus harbours the promoter (see below). This promoter drives the synthesis of an RNA transcript initiating at the boundary between the left hand U3 and R regions and terminating at the boundary between the right hand R and U5 region (Fig. 2). This RNA is packaged into retroviral particles and transported into the target cell to be infected. In the target cell the RNA genome is again reverse transcribed as described above.

[0035] According to the invention a retroviral vector is constructed in which the right- hand U3 region is altered (Fig. 3), but the normal left-hand U3 structure is main-

tained (Fig. 3); the vector can be normally transcribed into RNA utilizing the normal retroviral promoter located within the left-hand U3 region (Fig. 3). However the generated RNA will only contain the altered right-hand U3 structure. In the infected target cell, after reverse transcription, this altered U3 structure will be placed at both ends of the retroviral structure (Fig. 3).

[0036] If the altered region carries a polylinker (see below) instead of the U3 region then any promoter, including those directing tissue specific expression such as the WAP promoter (see below) can be easily inserted. This promoter will then be utilized exclusively in the target cell for expression of linked genes carried by the retroviral vector. Alternatively or additionally DNA segments homologous to one or more celluar sequences can be inserted into the polylinker for the purposes of gene targeting, by homologous recombination(see below).

[0037] According to the invention the term "polylinker" is used for a short stretch of artificially synthesized DNA which carries a number of unique restriction sites allowing the easy insertion of any promoter or DNA segment. The term "heterologous" is used for any combination of DNA sequences that is not normally found intimately associated in nature.

[0038] Gene expression is regulated by promoters. In the absence of promoter function a gene will not be expressed. The normal MLV retroviral promoter is fairly unselective in that it is active in most cell types (Majors, 1990). However a number of promoters exist that show activity only in very specific cell types. Such tissue-specific promoters will be the ideal candidates for the regulation of gene expression in retroviral vectors, limiting expression of the therapeutic genes to specific target cells.

[0039] In the packaging cell line the expression of the retroviral vector is regulated by the normal unselective retroviral promoter contained in the U3 region (Fig. 3). However as soon as the vector enters the target cell promoter conversion occurs, and the therapeutic or marker gene, e.g. b-galactosidase are expressed from a tissue specific promoter of choice introduced into the polylinker (Fig. 3). Not only can virtually any tissue specific promoter be included in the system, providing for the selective targeting of a wide variety of different cell types, but additionally, following the conversion event, the structure and properties of the retroviral vector no longer resembles that of a virus. This, of course, has extremely important consequences from a safety point of view, since ordinary or state of the art retroviral vectors readily undergo genetic recombination with the retroviral packaging construct and/or endogenous retroviruses to produce potentially pathogenic viruses. Promoter conversion (ProCon) vectors do not resemble retroviruses because they no longer carry U3 retroviral promoters after conversion thus reducing the possibility of genetic recombination.

[0040] The retroviral promoter structure is carried within the U3 region of the LTR. LTRs carry signals that allow them to integrate into the genome of the target cell. The integration of retroviral proviruses can also contribute to pathogenic changes (van Lohuizen and Berns, 1990). In one embodiment of the invention ProCon vectors can carry modified LTRs that no longer carry the signals required for integration. Again this increases the potential safety of these vector systems.

Gene Targeting

[0041] According to another aspect of the present invention the retroviral vector is used for targeted integration into the target cell. The integration of the proviral DNA version of the retroviral genome into the target cell is a major advance to the use of retroviruses as vectors when compared to other viruses such as adenoviruses, since it allows long term stable expression of transferred genes. However the random nature of this integration event also poses a major disadvantage to the use of retroviral vectors since it raises the possibility of insertional (in)activation of cellular tumour suppressor genes or proto-oncogenes and thus tumour induction (van Lohuizen and Berns, 1990).

[0042] Homologous recombination has been successfully used to target the integration of transfected or microinjected DNA to specific DNA loci and is routinely used in the construction of "knock-out" transgenic mice or animals (reviewed in Capecchi, 1989; Bradley *et al.*, 1992; Morrow and Kucherlapati, 1993). Unfortunately the efficiency of DNA transfer by such purely physical methods is extremely low. In contrast retroviral mediated gene transfer is very efficient, almost 100% of a population of cells being infectable. A combination of retroviral gene transfer with homologous recombination should allow the construction of an ideal system for locus targeted integration.

[0043] We have investigated the feasibility of introducing long homologous pieces of DNA into retroviral vectors in different locations to promote integration by homologous recombination (Saller,1994). Both gene conversion and homologous recombination have been evaluated. Using a cell line carrying a single copy of the HSV-tk gene as a target we have been able to disrupt the target at frequencies 15 fold higher than previously reported by others (Ellis and Bernstein, 1989). Cloning of the recombined fragments of DNA has revealed the presence of both target tk sequence and retroviral vector (Saller, 1994).

[0044] For targeted integration DNA segments homologous to cellular sequences are inserted into the polylinker of the ProCon vectors. After infection of the target cell and reverse transcription, these sequences will appear at the 5' terminal end of the provirus. Terminal homologies have been shown to favour homologous recombination (Bradley, 1991) to isogenic cellular sequences (Bradley, 1991). Infection of target cells which carry mutated versions of the homologous sequence

should result in the recombination and thus repair of the mutated sequence. Either just the homologous sequences will recombine into the cellular genome, or the complete vector will be inserted (Saller,1994). Not only has this vector class potential for use in gene repair, it can also be utilized to direct the integration of retroviral vectors carrying therapeutic genes to specific loci in the genome which are known not to harbour active genes. This will reduce considerably the possibility of insertional activation or inactivation as described above, and will thus contribute to the safety of the use of retroviral vectors.

[0045] The following examples will illustrate the invention further. These examples are however in no way intended to limit the scope of the present invention as obvious modifications will be apparent, and still other modifications and substitutions will be apparent to anyone skilled in the art.

[0046] The recombinant DNA methods employed in practicing the present invention are standard procedures, well known to those skilled in the art, and described in detail, for example, in "Molecular Cloning" (Sambrook et al. 1989) and in "A Practical Guide to Molecular Cloning" (Perbal, 1984).

[0047] Brief description of the drawings refered to in the following examples:

Fig. 1; Retroviral vector system
Fig. 2: Retroviral genome, reverse transcription
Fig. 3: ProCon principle
Fig. 4: PCR analysis, MLV probe
Fig. 5: PCR analysis, MMTV probe
Fig. 6: β-galactosidase expression in infected NIH and EF43 cells
Fig. 7: β-galactosidase expression in infected primary mammary glands cells from a pregnant mouse
Fig. 8: β-galactosidase expression after virus injection into mammary gland and the skin of a pregnant Balb/c mouse
Fig. 9: β-galactosidase expression in infected mammary tumour cells
Fig. 10: Targeted integration of a retroviral vector by homologous recombination

Example 1

Mammary gland specific expression after infection with ProCon Vectors carrying mammary specific promoters.

[0048] In the murine leukemia virus (MLV) retroviral vector known as BAG (Price et al., 1987) the β-galactosidase gene is driven by the promiscuous (i.e. non-tissue specific) MLV promoter in the U3 region of the LTR (Fig. 3). According to the present invention a derivative of the BAG vector has been constructed in which the MLV promoter (U3) located within the 3'LTR (Fig. 3) has been deleted and replaced with a polylinker, said polylinker allowing the facile introduction of heterologous promoters. The BAG vector lacking the U3 is expressed from the MLV promoter (U3) within the 5'LTR when introduced into a packaging cell line. As a result of the rearrangements occurring in the retroviral genome during its life cycle, following infection of its target cell, the polylinker will be duplicated at both ends of the retroviral genome as described above. Thereby a retroviral vector can be constructed in which the expression of the β-galactosidase gene of BAG will be controlled by any heterologous promoter inserted into the polylinker in the target cell (Fig. 3).

[0049] According to the principle set forth above the following specific promoters have been inserted into the polylinker region or the modified BAG vector:

[0050] Several subregions of the Mouse Mammary Tumour Virus (MMTV) promoter including a region that confers responsiveness to glucocorticoid hormones and a region containing an element that directs expression to the mammary gland.

[0051] The Whey Acidic Protein (WAP) promoter. This promoter controls the expression of WAP so that it is only produced in the mammary glands of pregnant and lactating rodents.

[0052] The control of the β-galactosidase gene expression by promoters inserted into the polylinker has been validated by infection studies using the constructed MMTV and WAP retroviral vectors to infect various cells.

[0053] To produce retroviral vector particles, the MMTV and WAP ProCon vectors have been transfected into the packaging cell line GP+E86 (Markowitz et al., 1988). After selection for neomycin resistance, which is encoded by the vector, stable populations and clones of recombinant ProCon virus producing cells were obtained. Virus containing supernatant from these populations was used to infect a mouse mammary cell line EF43 (Günzburg et al., 1988) as well as a mouse fibroblast cell line (Jainchill et al., 1969). Four days after infection the target cells were lysed and quantitative β-galactosidase assay revealed no expression in either cell type infected by the WAP carrying ProCon vectors and good expression in both cell types from the MMTV carrying ProCon vector (Fig. 6). This result is in accordance with the WAP promoter only functioning in vivo during late pregnancy and lactation and not in most simple in vitro mammary cell culture systems as represented by the EF43 cells. To investigate whether the WAP carrying ProCon vectors would be active in a complex primary mammary derived cell culture system, primary organoids from 8-10 day pregnant mice (Fig. 7) or from mammary tumours (Fig. 9) were taken into culture and infected with the supernatant from the same stably transfected population of transfected cell lines. Both ProCon vectors carrying the WAP and the MMTV promoter fragments were active in these primary cells (Fig. 7) and mammary tumour derived cells (Fig. 9) as demonstrated by β-galactosidase activity.

[0054] To investigate whether the WAP and MMTV carrying ProCon vectors were active in vivo and whether the expression of β-galactosidase was limited to the mammary gland in vivo, recombinant ProCon virus containing medium was injected in situ into the mammary glands or skin of 8-10 day pregnant mice. Five days later the mice were sacrificed, cell extracts prepared and a β-galactosidase assay performed. Both the WAP and MMTV fragment carrying ProCon vectors were expressed only in the pregnant mammary gland and not in the skin (cf M and S in Fig. 8). Thus in vivo the regulatory elements from both promoters limit expression to the mammary gland whereas in vitro the regulatory elements from the WAP promoter retain their strict tissue specificity but those of MMTV do not.

[0055] These ProCon vectors carrying tissues specific promoters and regulatory elements will be useful for directing the expression of therapeutic genes to predefined cell types, tissues and organs. Potential therapeutic genes include melittin, which has anti-HIV and antitumour effects, and genes which prime cells for death including the thymidine kinase, guanine phosphoribosytransferase and cytosine deaminase genes, cytochrome P450 as well as genes involved in cell cycle regulation such as SDI/WAF-1/CIP-1.

Example 2

Validation of promoter conversion in cells infected with a ProCon vector that originally carried the MMTV promoter in the 3'LTR.

[0056] A ProCon vector carrying the promoter region from mouse mammary tumour virus (MMTV) was transfected into a packaging cell line and the resultant recombinant vector particles used to infect an established human bladder carcinoma cell line (EJ). Infected cell clones were selected in medium carrying the neomycin analog G418 (since the vector carries a neomycin resistance gene driven from an internal SV40 promoter). DNA was prepared from one of the infected clones and nontransfected parental EJ cells and used for Polymerase Chain Reactions (PCR). The PCRs were performed using one of two primers that specifically recognise and bind to MMTV sequences (A, B in Figs. 4 & 5) or the MLV R region (C in Fig. 4) of the LTR together with a primer located within the marker gene (Figs. 4 & 5). Since the marker gene primer is only located down stream of the MMTV (or MLV R region) sequence if promoter conversion has occurred, a positive PCR signal obtained with the MMTV primers in combination with the marker gene primer is indicative of this. In Fig. 4 the PCR products using primers A, B or C are shown after hybridization to a labelled fragment from the MLV sequence, verifying that all three PCR products are of MLV origin. The size of the fragments verifies that promoter conversion has occured. Fig. 5 shows the PCR products using primer A or B and hybdridized to an MMTV specific probe, again verifying that promoter conversion has occured.

Example 3

Construction of ProCon Vectors for targeted integration.

[0057] Using the same BAG vector described in Example 1 above, a retroviral vector can be constructed in which a DNA sequence with homology to a cellular sequence can be inserted into the LTR. The resulting vector can be used to target the integration of either the homologous sequence inserted into the vector or the whole or part of the vectors into the homologous sequence present in the host cell genome.

[0058] According to the principle set forth above, a fragment of the thymidine kinase (tk) gene of herpes simplex virus (HSV) has been inserted into the polylinker region of the modified BAG vector (tk mutant in Fig. 10, Saller, 1994).

[0059] A cell line has also been established that has no functional copy of the mammalian tk gene and instead carries one copy of the HSV-tk gene (Saller, 1994). This cell line has been infected with the tk carrying BAG vector and cells that have undergone disruption of the HSV-tk gene have been selected (Fig. 10).

[0060] The above examples have illustrated the principles and consequences of the promoter conversion vectors provided by the present invention.

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Claims

1. A retroviral vector which undergoes promoter conversion comprising a 5' long terminal repeat region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' long terminal repeat region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a sequence comprising a heterologous promoter not related to the retroviral vector, said promoter regulating, after infection of the target cell, expression of at least one of the coding sequences, said coding sequences being inserted into the body of the vector.

2. A retroviral vector according to claim 1, wherein deleted U3 region comprises a regulatory element.

3. A retroviral vector according to claim 1 or 2, wherein said regulatory element and/or promoter are target cell specific in their expression.

4. A retroviral vector according to claim 3, wherein said target cell specific regulatory element and promoter are selected from one or more elements of the group consisting of WAP, MMTV, b-lactoglobulin and casein specific regulatory elements and promoters, pancreas specific regulatory elements and promoters including carbonic anhydrase II and b-glucokinase regulatory elements and promoters, lymphocyte specific regulatory elements and promoters including immunoglobulin and MMTV lymphocytic specific regulatory elements and promoters and MMTV specific regulatory elements and promoters conferring responsiveness to glucocorticoid hormones or directing expression to the mammary gland.

5. A retroviral vector according to anyone of claims 2 to 4, wherein said regulatory element and promoter regulate the expression of at least one of the coding sequences of said retroviral vector.

6. A retroviral vector according to anyone of claims 1 to 5, wherein said LTR regions are selected from at least one element of the group consisting of LTRs of MLV, MMTV, MSV, SIV, HIV, HTLV, FIV, FeLV, BLV and MPMV.

7. A retroviral vector according to anyone of claims 1 to 6, wherein said retroviral vector is a BAG vector.

8. A retroviral vector according to anyone of claims 1 to 7, wherein said coding sequence is selected from one or more elements of the group consisting of marker genes, therapeutic genes, antiviral genes, antitumour genes, cytokine genes.

9. A retroviral vector according to claim 8, wherein said marker or therapeutic gene is selected from one or more elements of the group consisting of b-galactosidase gene and neomycin gene, Herpes Simplex Virus, thymidine kinase gene, puromycin gene, cytosine deaminase gene, hygromycin gene, secreted alkaline phosphatase gene, guanine phosphoribosyl transferase (gpt) gene, alcohol dehydrogenase gene and hypoxanthine phosphoribosyl transferase (HPRT) gene.

10. A retroviral vector according to anyone of claims 1 to 9, wherein at least one of said coding sequences for a retroviral protein is altered or at least partially deleted.

11. A retroviral vector according to anyone of claims 1 to 10, wherein retroviral sequences involved in integration of retroviruses are altered or at least partially deleted.

12. A retroviral vector according to anyone of claims 1 to 11, wherein said heterologous DNA fragment is homologous to one or more cellular sequences or a part thereof.

13. A retroviral vector according to anyone of claims 1 to 12, wherein said regulator elements are regulatable by transacting molecules.

14. A retroviral vector system comprising a retroviral vector according to anyone of claims 1 to 13 as a first component; and
a packaging cell line harbouring at least one retroviral or recombinant retroviral construct coding for proteins required for said retroviral vector to be packaged.

15. A retroviral vector system according to claim 14 wherein the packaging cell line harbours retroviral or recombinant retroviral constructs coding for those retroviral proteins which are not encoded in said retroviral vector according to anyone of claims 1 to 13.

16. A retroviral vector system according to claim 14 or 15 wherein the packaging cell line is selected from the group consisting of psi-2, psi-Crypt, psi-AM, GP+E-86, PA317 and GP+envAM-12.

17. Use of a retroviral vector system according to claims 14-16 for the production of a pharmaceutical composition for introducing homologous or heterologous nucleotide sequences into human or animal cells *in vitro* and *in vivo* comprising transfecting a packaging cell line of a retroviral vector system according to anyone of claims 14 to 16 with a retroviral vector according to anyone of claims 1 to 13, and infecting a target cell population with said recombinant retroviruses produced by the packaging cell line.

18. Use according to claim 17, wherein the nucleotide sequences are selected from one or more elements of the group consisting of genes or parts of genes encoding for proteins, regulatory sequences and promoters.

19. A retroviral provirus produced by replicating the retroviral vector of anyone of claims 1 to 13 in a retroviral vector system according to anyone of claims 14 to 16 wherein said heterologous promoter inserted in said deleted U3 region become duplicated during the process of reverse transcription in the infected target cell and appear in the 5'LTR as well as in the 3'LTR of the resulting provirus.

20. Use of a retroviral vector according to anyone of claims 1 to 13 for producing a pharmaceutical composition for gene therapy in mammals including humans.

21. Use of a retroviral vector system according to anyone of claims 14 to 16 for producing a pharmaceutical composition for gene therapy in mammals including humans.

22. Use of a retroviral provirus according to claim 19 for producing a pharmaceutical composition for gene therapy in mammals including humans.

23. Use of a retroviral vector according to anyone of claims 1 to 13 for the manufacture of a pharmaceutical composition for targeted integration in said homologous cellular sequences.

24. Use of a retroviral vector system according to anyone of claims 14 to 16 for the manufacture of a pharmaceutical composition for targeted integration in said homologous cellular sequences.

25. Use of a retroviral provirus according to claim 19 for the manufacture of a pharmaceutical composition for targeted integration in said homologous cellular sequences.

26. mRNA of a retroviral provirus according to claim 19.

27. RNA of a retroviral vector according to anyone of claims 1 to 13.

28. Recombinant retroviral particle obtained by transfecting a packaging cell line of a retroviral vector system according to anyone of claims 14-16 with a retroviral vector according to anyone of claims 1 to 13, and culturing the cells under suitable conditions.

29. Pharmaceutical composition containing a therapeutically effective amount of a recombinant retroviral particle according to claim 28.

Patentansprüche

1. Ein retroviraler Vektor, der Promotorkonversion durchmacht, umfassend eine 5'Long Terminal Repeat Region der Struktur U3-R-U5; eine oder meh-

rere Sequenzen, die von kodierenden und nicht-kodierenden Sequenzen ausgewählt sind; und eine 3'Long Terminal Repeat Region, die eine vollständig oder teilweise deletierte U3 Region umfaßt, wobei jene deletierte U3 Region durch eine Sequenz ersetzt wurde, die einen heterologen, nicht mit dem retroviralen Vektor verwandten Promotor umfaßt, jener Promotor nach der Infektion der Zielzelle die Expression von mindestens einer der kodierenden Sequenzen reguliert und die kodierenden Sequenzen in den Körper des Vektors eingefügt sind.

2. Ein retroviraler Vektor nach Anspruch 1, wobei jene deletierte U3 Region ein regulatorisches Element enthält.

3. Ein retroviraler Vektor nach Anspruch 1 oder 2, wobei das regulatorische Element und/oder der Promotor in ihrer Expression Zielzellen-spezifisch sind.

4. Ein retroviraler Vektor nach Anspruch 3, wobei das Zielzellen-spezifische, regulatorische Element und der Promotor von einem oder mehreren Elementen aus der Gruppe ausgewählt wurden, bestehend aus WAP, MMTV, β-Lactoglobulin und Casein spezifischen regulatorischen Elementen und Promotoren, Pankreas spezifischen regulatorischen Elementen und Promotoren, einschließlich Carboanhydrase II und β-Glucokinase regulatorischen Elementen und Promotoren, Lymphozyten spezifischen regulatorischen Elementen und Promotoren einschließlich Immunglobulin und MMTV Lymphozyten spezifischen regulatorischen Elementen und Promotoren und MMTV spezifischen regulatorischen Elementen und Promotoren, die Reaktionsfähigkeit gegenüber Glucocorticoidhormonen verleihen oder die Expression auf die Brustdrüsen richten.

5. Ein retroviraler Vektor nach einem der Ansprüche 2 bis 4, wobei das regulatorische Element und der Promotor die Expression von mindestens einer der kodierenden Sequenzen des retroviralen Vektors regulieren.

6. Ein retroviraler Vektor nach einem der Ansprüche 1 bis 5, wobei die LTR Regionen von mindestens einem Element aus der Gruppe, die aus LTRs von MLV, MMTV, MSV, SIV, HIV, HTLV, FIV, FeLV, BLV und MPMV besteht, ausgewählt sind.

7. Ein retroviraler Vektor nach einem der Ansprüche 1 bis 6, wobei der retrovirale Vektor ein BAG Vektor ist.

8. Ein retroviraler Vektor nach einem der Ansprüche 1 bis 7, wobei die kodierende Sequenz von einem oder mehreren Elementen aus der Gruppe, die aus Markergenen, therapeutischen Genen, antiviralen Genen, Antitumorgenen, Cytokingenen besteht, ausgewählt sind.

9. Ein retroviraler Vektor nach Anspruch 8, wobei das Markergen oder therapeutische Gen von einem oder mehreren Elementen aus der Gruppe ausgewählt sind, bestehend aus β-Galactosidasegen und Neomycingen, Herpes Simplex Virus Thymidinkinasegen, Puromycingen, Cytosindeaminasegen, Hygromycingen, Gen der sekretierten alkalischen Phosphatase, Guanin-Phosphoribosyltransferasegen (gpt), Alkoholdehydrogenasegen und Hypoxanthin-Phosphoribosyltransferasegen (HPRT).

10. Ein retroviraler Vektor nach einem der Ansprüche 1 bis 9, wobei mindestens eine der für ein retrovirales Protein kodierenden Sequenzen verändert oder mindestens teilweise deletiert ist.

11. Ein retroviraler Vektor nach einem der Ansprüche 1 bis 10, wobei retrovirale Sequenzen, die an der Integration von Retroviren beteiligt sind, verändert oder mindestens teilweise deletiert sind.

12. Ein retroviraler Vektor nach einem der Ansprüche 1 bis 11, wobei das heterologe DNA-Fragment zu einem oder mehreren zellulären Sequenzen oder einem Teil davon homolog ist.

13. Ein retroviraler Vektor nach einem der Ansprüche 1 bis 12, wobei die regulatorischen Elemente durch *in trans* wirkende Moleküle regulierbar sind.

14. Ein retrovirales Vektorsystem umfassend als ersten Bestandteil einen retroviralen Vektor nach einem der Ansprüche 1 bis 13; und eine Verpackungszellinie, die mindestens ein retrovirales oder rekombinant retrovirales Konstrukt beherbergt, das für die zur Verpackung des retroviralen Vektors notwendigen Proteine kodiert.

15. Ein retrovirales Vektorsystem nach Anspruch 14, wobei die Verpackungszellinie retrovirale oder rekombinant retrovirale Konstrukte beherbergt, die für diejenigen retroviralen Proteine kodieren, die nicht von dem retroviralen Vektor nach einem der Ansprüche 1 bis 13 kodiert werden.

16. Ein retrovirales Vektorsystem nach Anspruch 14 oder 15, wobei die Verpackungszellinie aus der Gruppe, die aus psi-2, psi-Crypt, psi-AM, GP+E-86, PA317 und GP+envAM-12 besteht, ausgewählt ist.

17. Verwendung eines retroviralen Vektorsystems nach den Ansprüchen 14 bis 16 zum Herstellen einer pharmazeutischen Zusammensetzung zur Einführung homologer oder heterologer Nukleotidsequenzen in menschlichen oder tierischen Zellen *in vitro*

und *in vivo,* umfassend das Transfizieren einer Verpackungszellinie eines retroviralen Vektorsystems nach einem der Ansprüche 14 bis 16 mit einem retroviralen Vektor nach einem der Ansprüche 1 bis 13 und das Infizieren einer Zielzellpopulation mit den durch die Verpackungszellinie hergestellten, rekombinanten Retroviren.

18. Verwendung nach Anspruch 17, wobei die Nukleotidsequenzen von einem oder mehreren Elementen aus der Gruppe, die aus für Proteine kodierenden Genen oder Teilen von Genen, regulatorischen Sequenzen und Promotoren besteht, ausgewählt sind.

19. Ein retroviraler Provirus, der durch Replizieren des retroviralen Vektors nach einem der Ansprüche 1 bis 13 in einem retroviralen Vektorsystem nach einem der Ansprüche 14 bis 16 hergestellt wurde, wobei der in die deletierte U3 Region eingefügte, heterologe Promotor während des Vorgangs der reversen Transkription in der infizierten Zielzelle dupliziert wird und in der 5'LTR und in der 3'LTR des resultierenden Provirus erscheint.

20. Verwendung eines retroviralen Vektors nach einem der Ansprüche 1 bis 13 zum Herstellen einer pharmazeutischen Zusammensetzung zur Gentherapie bei Säugetieren einschließlich Menschen.

21. Verwendung eines retroviralen Vektorsystems nach einem der Ansprüche 14 bis 16 zum Herstellen einer pharmazeutischen Zusammensetzung zur Gentherapie bei Säugetieren einschließlich Menschen.

22. Verwendung eines retroviralen Provirus nach Anspruch 19 zum Herstellen einer pharmazeutischen Zusammensetzung zur Gentherapie bei Säugetieren einschließlich Menschen.

23. Verwendung eines retroviralen Vektors nach einem der Ansprüche 1 bis 13 zur Herstellung einer pharmazeutischen Zusammensetzung zur gezielten Integration in die homologen zellulären Sequenzen.

24. Verwendung eines retroviralen Vektorsystems nach einem der Ansprüche 14 bis 16 zur Herstellung einer pharmazeutischen Zusammensetzung zur gezielten Integration in die homologen zellulären Sequenzen.

25. Verwendung eines retroviralen Provirus nach Anspruch 19 zur Herstellung einer pharmazeutischen Zusammensetzung zur gezielten Integration in die homologen zellulären Sequenzen.

26. mRNA eines retroviralen Provirus nach Anspruch 19.

27. RNA eines retroviralen Vektors nach einem der Ansprüche 1 bis 13.

28. Rekombinante, retrovirale Partikel, die durch Transfektion einer Verpackungszellinie eines retroviralen Vektorsystems nach einem der Ansprüche 14 bis 16 mit einem retroviralen Vektor nach einem der Ansprüche 1 bis 13 und Kultivieren der Zellen unter geeigneten Bedingungen erhalten werden.

29. Pharmazeutische Zusammensetzung enthaltend eine therapeutisch wirksame Menge eines rekombinanten, retroviralen Partikels nach Anspruch 28.

Revendications

1. Vecteur rétroviral qui subit une conversion de promoteur, comprenant une région de longue répétition terminale 5' de structure U3-R-U5 ; une ou plusieurs séquences sélectionnées parmi des séquences codantes et non codantes ; et une région de longue répétition terminale en 3' comprenant une région U3 complètement ou partiellement délétée, ladite région U3 délétée étant remplacée par une séquence comprenant un promoteur hétérologue qui n'est pas apparenté au vecteur rétroviral, ledit promoteur régulant, après infection de la cellule cible, l'expression d'au moins une des séquences codantes, ladite séquence codante étant insérée dans le corps du vecteur.

2. Vecteur rétroviral selon la revendication 1, dans lequel ladite région U3 délétée comprend un élément de régulation.

3. Vecteur rétroviral selon la revendication 1 ou 2, dans lequel ledit élément de régulation et/ou promoteur sont spécifiques de cellules cibles pour leur expression.

4. Vecteur rétroviral selon la revendication 3, dans lequel ledit élément de régulation et le promoteur spécifique d'une cellule cible sont choisis parmi un ou plusieurs éléments de l'ensemble constitué d'éléments de régulation et de promoteurs de WAP, MMTV, β-lactoglobuline et caséine, d'éléments de régulation et de promoteurs spécifiques du pancréas incluant l'anhydrase carbonique II et d'éléments de régulation et de promoteurs de la β-glucokinase, d'éléments de régulation et de promoteurs spécifiques des lymphocytes incluant les éléments de régulation et les promoteurs spécifiques lymphocytiques d'immunoglobulines et de MMTV et les éléments de régulation et promoteurs spécifiques de MMTV qui confèrent une capacité de réponse aux hormones glucocorticoïdiques ou qui dirigent l'expression dans la glande mammaire

5. Vecteur rétroviral selon l'une quelconque des revendications 2 à 4, dans lequel ledit élément de régulation et ledit promoteur régulent l'expression d'au moins une des séquences codantes dudit vecteur rétroviral.

6. Vecteur rétroviral selon l'une quelconque des revendications 1 à 5, dans lequel lesdites régions LTR sont sélectionnées parmi au moins un élément de l'ensemble consistant en LTR de MLV, MMTV, MSV, SIV, HIV, HTLV, FIV, FeLV, BLV et MPMV.

7. Vecteur rétroviral selon l'une quelconque des revendications 1 à 6, dans lequel ledit vecteur rétroviral est un vecteur BAG.

8. Vecteur rétroviral selon l'une quelconque des revendications 1 à 7, dans lequel ladite séquence codante est choisie parmi un ou plusieurs éléments de l'ensemble consistant en gènes marqueurs, gènes thérapeutiques, gènes antiviraux, gènes antitumoraux, gènes de cytokine.

9. Vecteur rétroviral selon la revendication 8, dans lequel ledit gène marqueur ou thérapeutique est choisi parmi un ou plusieurs éléments de l'ensemble consistant en gène de β-galactosidase et gène de néomycine, gène de thymidine kinase du virus de l'herpès, gène de puromycine, gène de cytosine désaminase, gène d'hygromycine, gène de phosphatase alcaline sécrétée, gène de guanine phosphoribosyltransférase (gpt), gène d'alcool déshydrogénase, et gène d'hypoxanthine phosphoribosyltransférase (HPRT).

10. Vecteur rétroviral selon l'une quelconque des revendications 1 à 9, dans lequel au moins une desdites séquences codant une protéine rétrovirale est altérée ou au moins partiellement délétée.

11. Vecteur rétroviral selon l'une quelconque des revendications 1 à 10, dans lequel les séquences rétrovirales impliquées dans l'intégration de rétrovirus sont altérées ou au moins partiellement délétées.

12. Vecteur rétroviral selon l'une quelconque des revendications 1 à 11, dans lequel ledit fragment d'ADN hétérologue est homologue d'une ou plusieurs séquences cellulaires ou d'une partie de celles-ci.

13. Vecteur rétroviral selon l'une quelconque des revendications 1 à 12, dans lequel lesdits éléments de régulation peuvent être régulés par des molécules agissant en trans.

14. Système de vecteur rétroviral comprenant un vecteur rétroviral selon l'une quelconque des revendications 1 à 13 comme premier composant ; et une lignée cellulaire d'encapsidation abritant au moins une construction rétrovirale ou rétrovirale recombinée codant des protéines nécessaires pour que ledit vecteur rétroviral soit encapsidé.

15. Système de vecteur rétroviral selon la revendication 14, dans lequel la lignée cellulaire d'encapsidation abrite des constructions rétrovirales ou rétrovirales recombinées codant les protéines rétrovirales qui ne sont pas codées dans ledit vecteur rétroviral selon l'une quelconque des revendications 1 à 13.

16. Système de vecteur rétroviral selon la revendication 14 ou 15, dans lequel la lignée cellulaire d'encapsidation est choisie dans l'ensemble consistant en psi-2, psi-Crypt, psi-AM, GP+E-86, PA317 et GP+envAM-12.

17. Utilisation d'un système de vecteur rétroviral selon les revendications 14-16 pour la production d'une composition pharmaceutique pour l'introduction de séquences nucléotidiques homologues ou hétérologues dans des cellules humaines ou animales *in vitro* et *in vivo*, comprenant les étapes consistant à transfecter une lignée cellulaire d'encapsidation d'un système de vecteur rétroviral selon l'une quelconque des revendications 14 à 16 par un vecteur rétroviral selon l'une quelconque des revendications 1 à 13, et à infecter une population de cellules cibles par lesdits rétrovirus recombinés produits par la lignée cellulaire d'encapsidation.

18. Utilisation selon la revendication 17, dans laquelle les séquences nucléotidiques sont choisies parmi un ou plusieurs éléments de l'ensemble consistant en gènes ou en parties de gènes codant pour des protéines, des séquences de régulation, et des promoteurs.

19. Provirus rétroviral produit par réplication du vecteur rétroviral de l'une quelconque des revendications 1 à 13 dans un système de vecteur rétroviral selon l'une quelconque des revendications 14 à 16, dans lequel ledit promoteur hétérologue inséré dans ladite région U3 délétée est dupliqué pendant le processus de transcription inverse dans la cellule cible infectée et apparaît dans la 5'LTR ainsi que dans le 3'LTR du provirus obtenu.

20. Utilisation d'un vecteur rétroviral selon l'une quelconque des revendications 1 à 13 pour la production d'une composition pharmaceutique pour la thérapie génique chez des mammifères incluant l'homme.

21. Utilisation d'un système de vecteur rétroviral selon

l'une quelconque des revendications 14 à 16 pour la production d'une composition pharmaceutique pour la thérapie génique chez des mammifères incluant l'homme.

22. Utilisation d'un provirus rétroviral selon la revendication 19 pour la production d'une composition pharmaceutique pour la thérapie génique chez des mammifères incluant l'homme.

23. Utilisation d'un vecteur rétroviral selon l'une quelconque des revendications 1 à 13, pour la fabrication d'une composition pharmaceutique pour l'intégration ciblée dans lesdites séquences cellulaires homologues.

24. Utilisation d'un système de vecteur rétroviral selon l'une quelconque des revendications 14 à 16, pour la fabrication d'une composition pharmaceutique pour l'intégration ciblée dans lesdites séquences cellulaires homologues.

25. Utilisation d'un provirus rétroviral selon la revendication 19, pour la fabrication d'une composition pharmaceutique pour l'intégration ciblée dans lesdites séquences cellulaires homologues.

26. ARNm d'un provirus rétroviral selon la revendication 19.

27. ARN d'un vecteur rétroviral selon l'une quelconque des revendications 1 à 13.

28. Particules rétrovirales recombinées obtenues par transfection une lignée cellulaire d'encapsidation d'un système de vecteur rétroviral selon l'une quelconque des revendications 14 à 16 par un vecteur rétroviral selon l'une quelconque des revendications 1 à 13, et culture des cellules dans des conditions appropriées.

29. Composition pharmaceutique contenant une quantité thérapeutiquement efficace d'une particule rétrovirale recombinée selon la revendication 28.

Fig.1

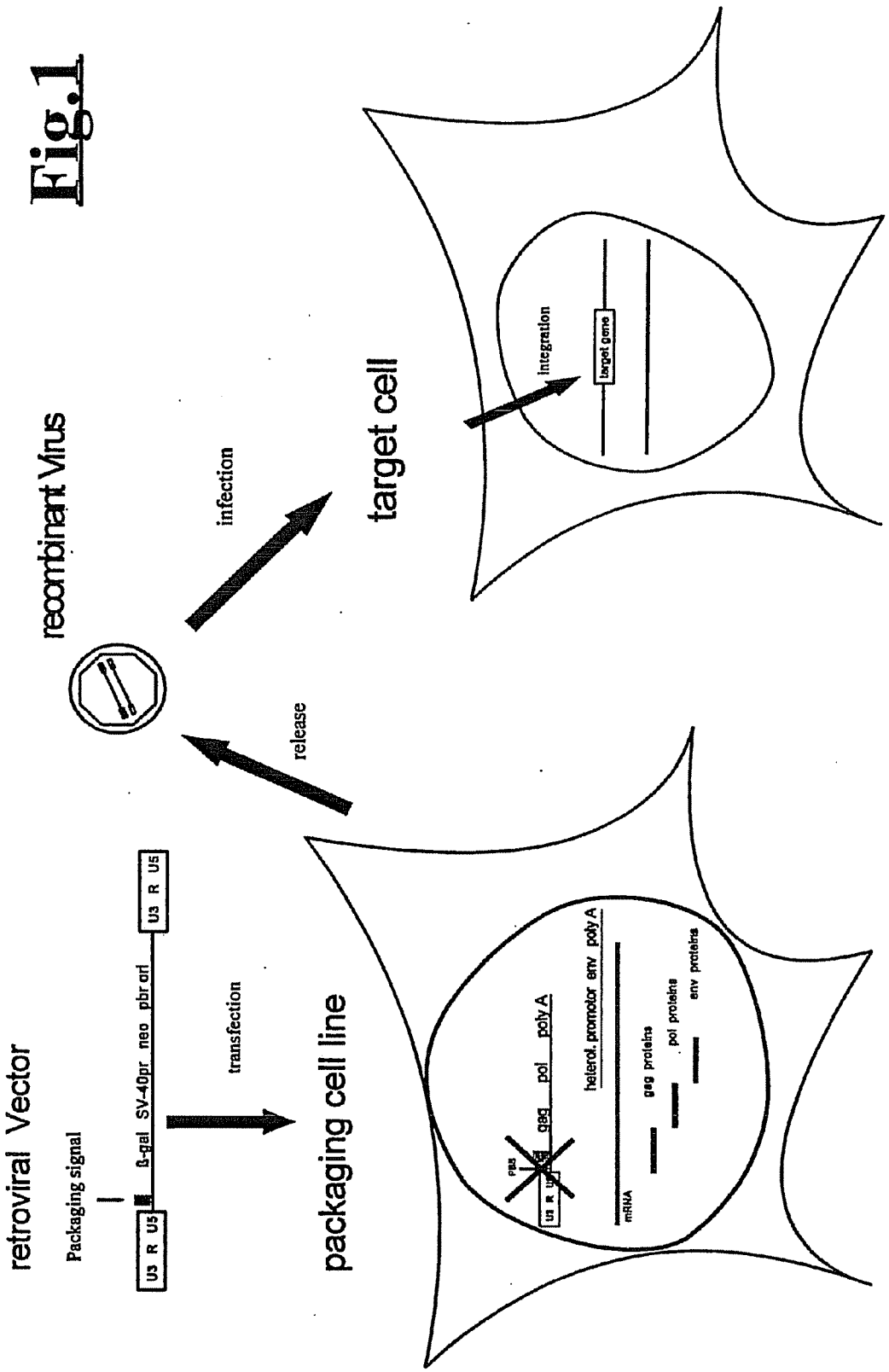

retroviral Vector
Packaging signal
U3 R U5
β-gal SV-40pr neo pbr ori
U3 R U5
transfection
packaging cell line
release
recombinant Virus
infection
target cell
integration
target gene
PBS
gag
pol
poly A
heterol. promotor env poly A
mRNA
gag proteins
pol proteins
env proteins

Fig. 2

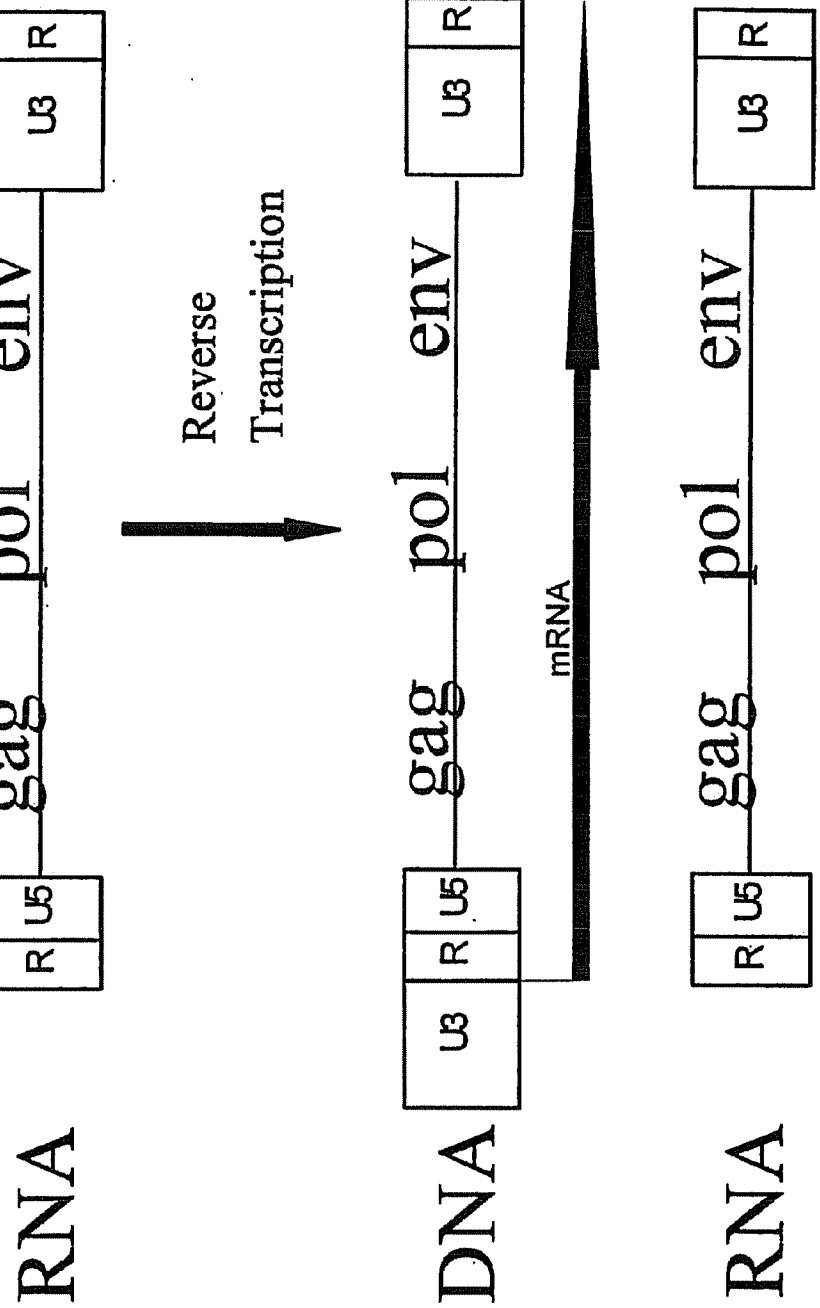

RNA
R
U5
gag
pol
env
U3
R
Reverse
Transcription
DNA
U3
R
U5
gag
pol
env
U3
R
U5
mRNA
RNA
R
U5
gag
pol
env
U3
R

Fig. 3

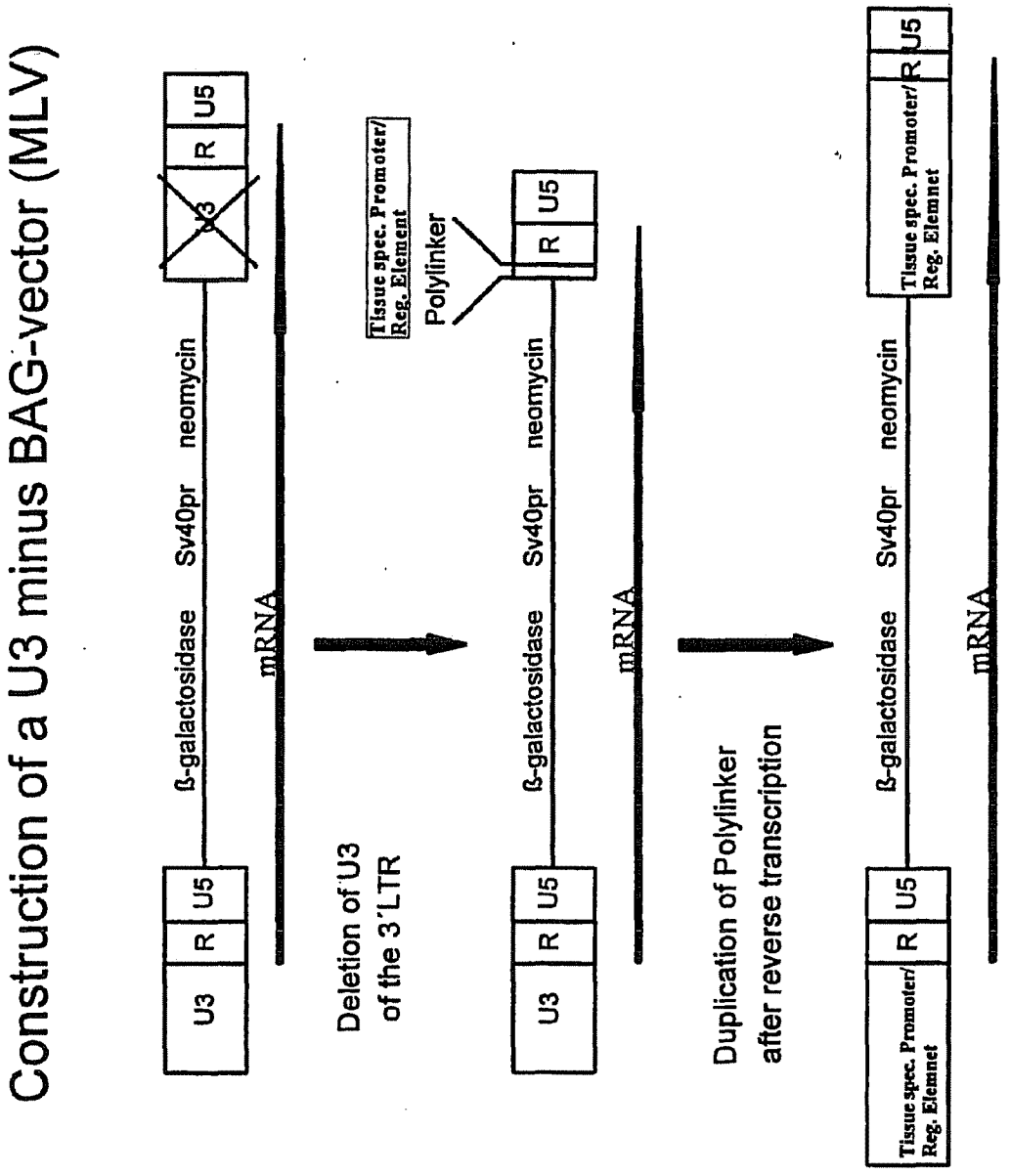
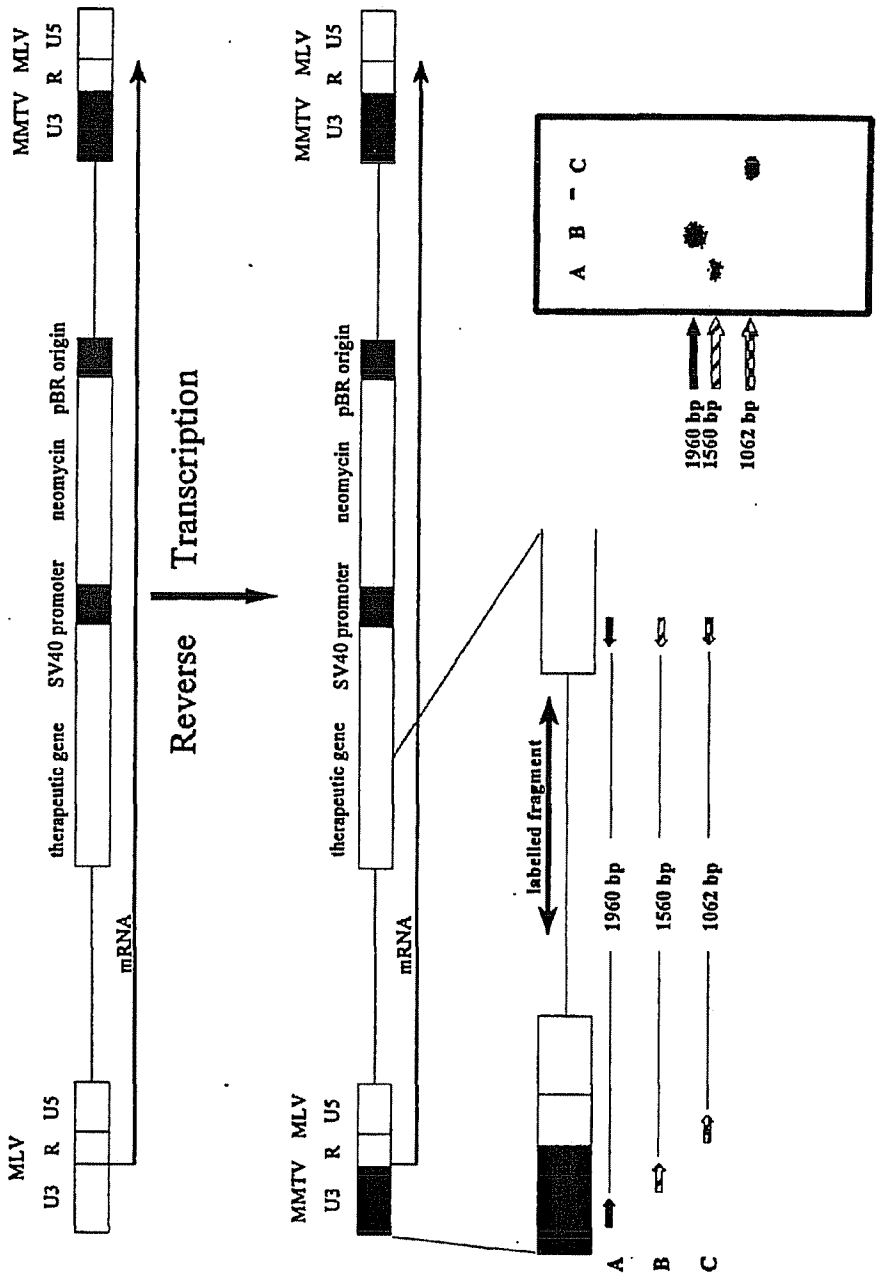

Construction of a U3 minus BAG-vector (MLV)
U3
R
U5
ß-galactosidase
Sv40pr
neomycin
mRNA
Deletion of U3
of the 3'LTR
Tissue spec. Promoter/
Reg. Element
Polylinker
Duplication of Polylinker
after reverse transcription

Fig. 4


MMTV MLV
U3 R U5
therapeutic gene
SV40 promoter
neomycin
pBR origin
mRNA
MLV
U3 R U5
Reverse
Transcription
MMTV MLV
U3 R U5
therapeutic gene
SV40 promoter
neomycin
pBR origin
mRNA
MMTV MLV
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C
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1560 bp
1062 bp

Fig. 5

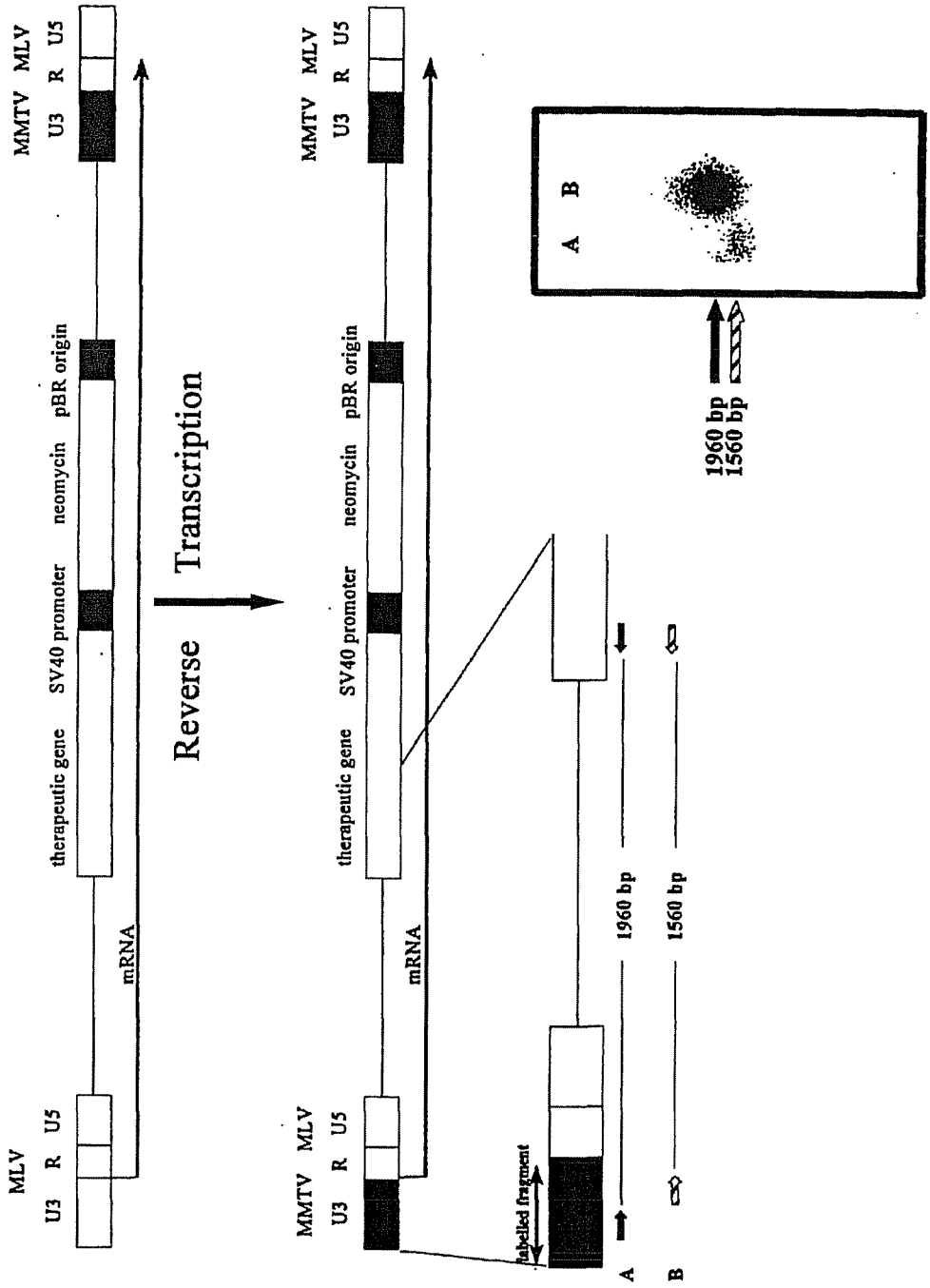
MMTV MLV
U3 R U5
therapeutic gene
SV40 promoter
neomycin
pBR origin
mRNA
Transcription
Reverse
MLV
U3 R U5
MMTV MLV
U3 R U5
therapeutic gene
SV40 promoter
neomycin
pBR origin
mRNA
MMTV MLV
U3 R U5
labelled fragment
A
B
1960 bp
1560 bp
A B
1960 bp
1560 bp

Fig. 6

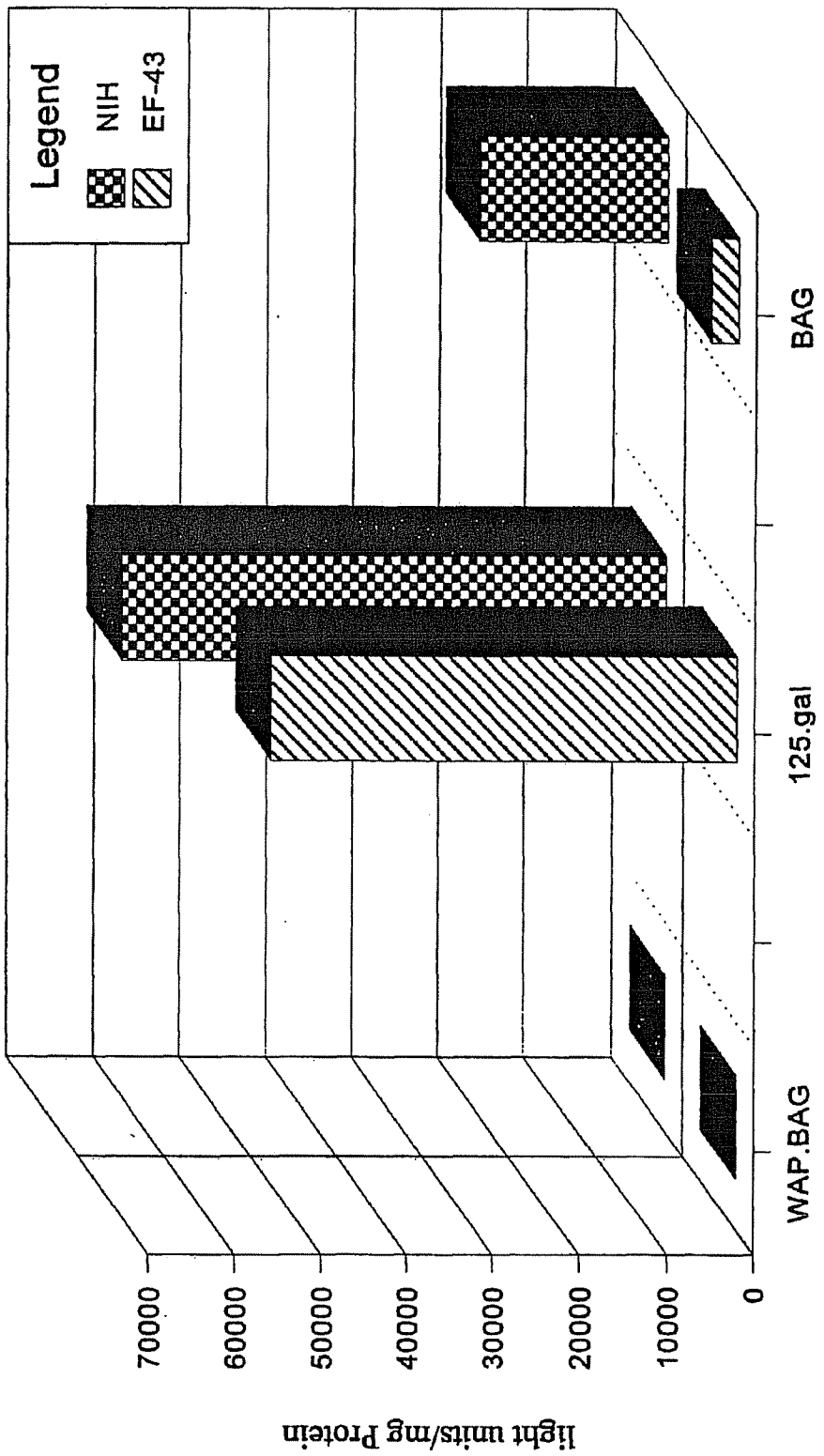
Legend
NIH
EF-43
light units/mg Protein
70000
60000
50000
40000
30000
20000
10000
0
WAP.BAG
125.gal
BAG

Fig. 7

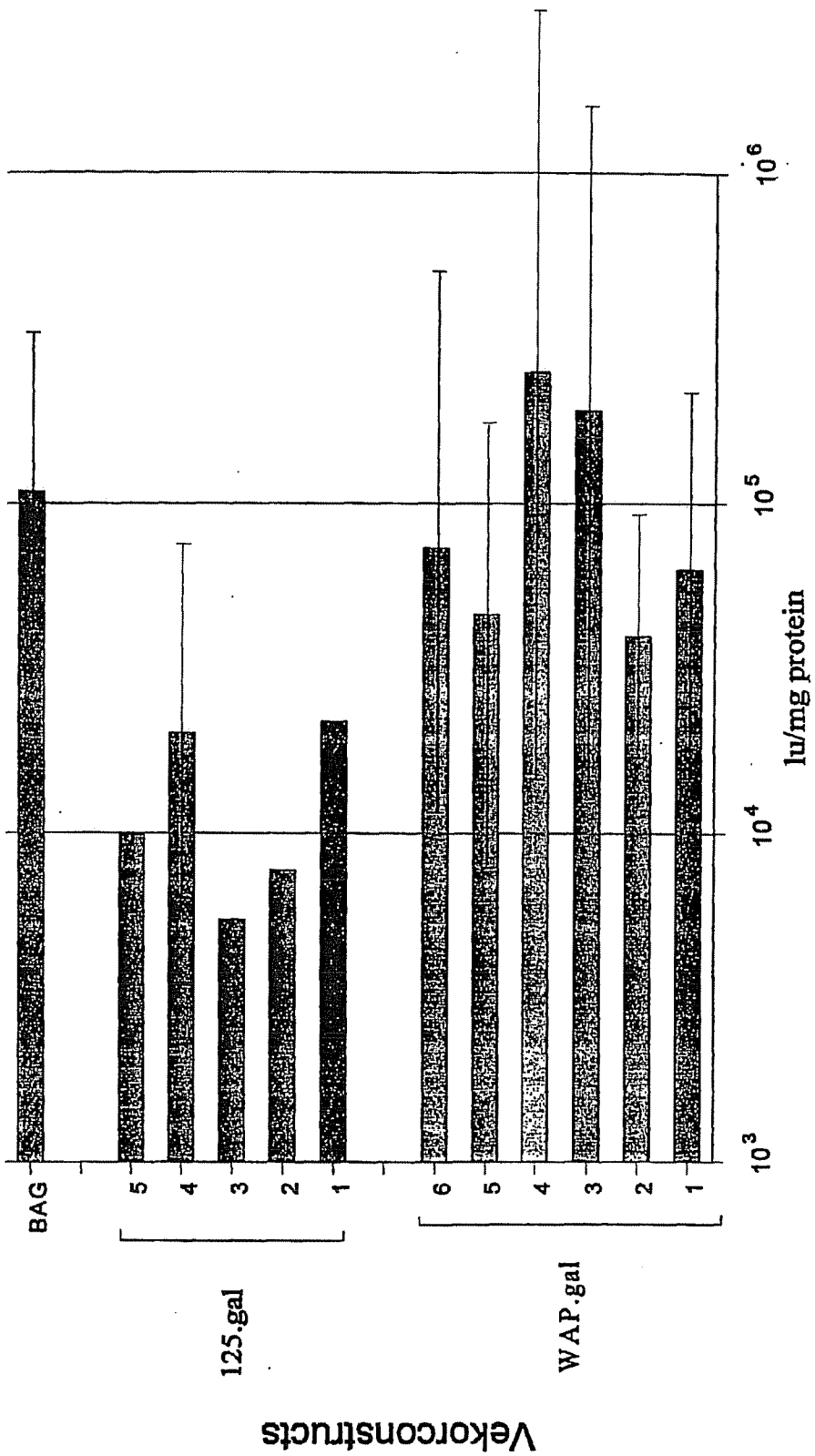
lu/mg protein
10^3
10^4
10^5
10^6
BAG
5
4
3
2
1
125.gal
6
5
4
3
2
1
WAP.gal
Vekorconstructs

Fig. 8

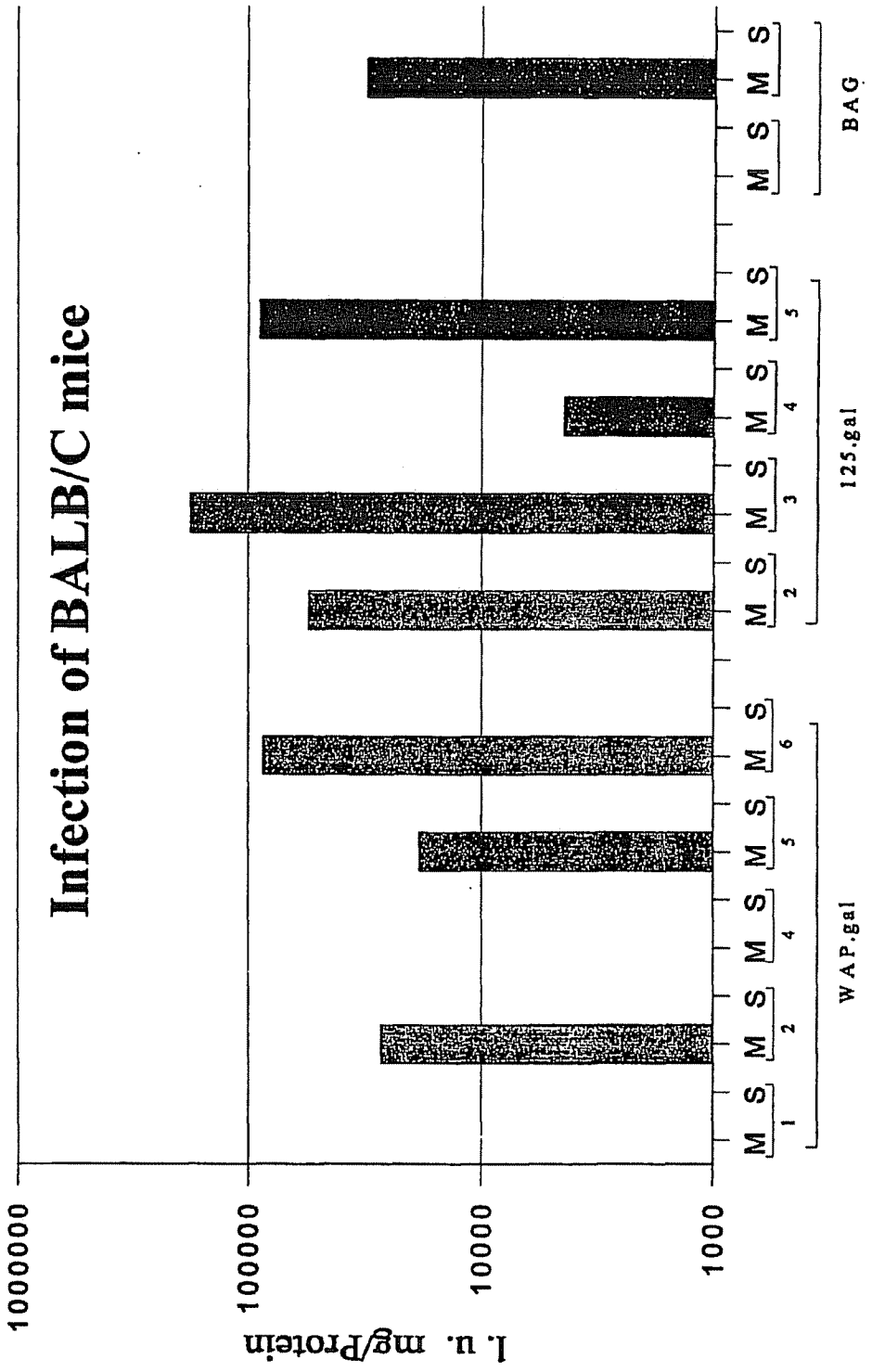
Infection of BALB/C mice
l. u. mg/Protein
1000000
100000
10000
1000
M S M S M S M S M S M S M S M S M S M S M S M S
1 2 3 4 5 6 2 3 4 5
WAP.gal
125.gal
BAG

Fig. 9

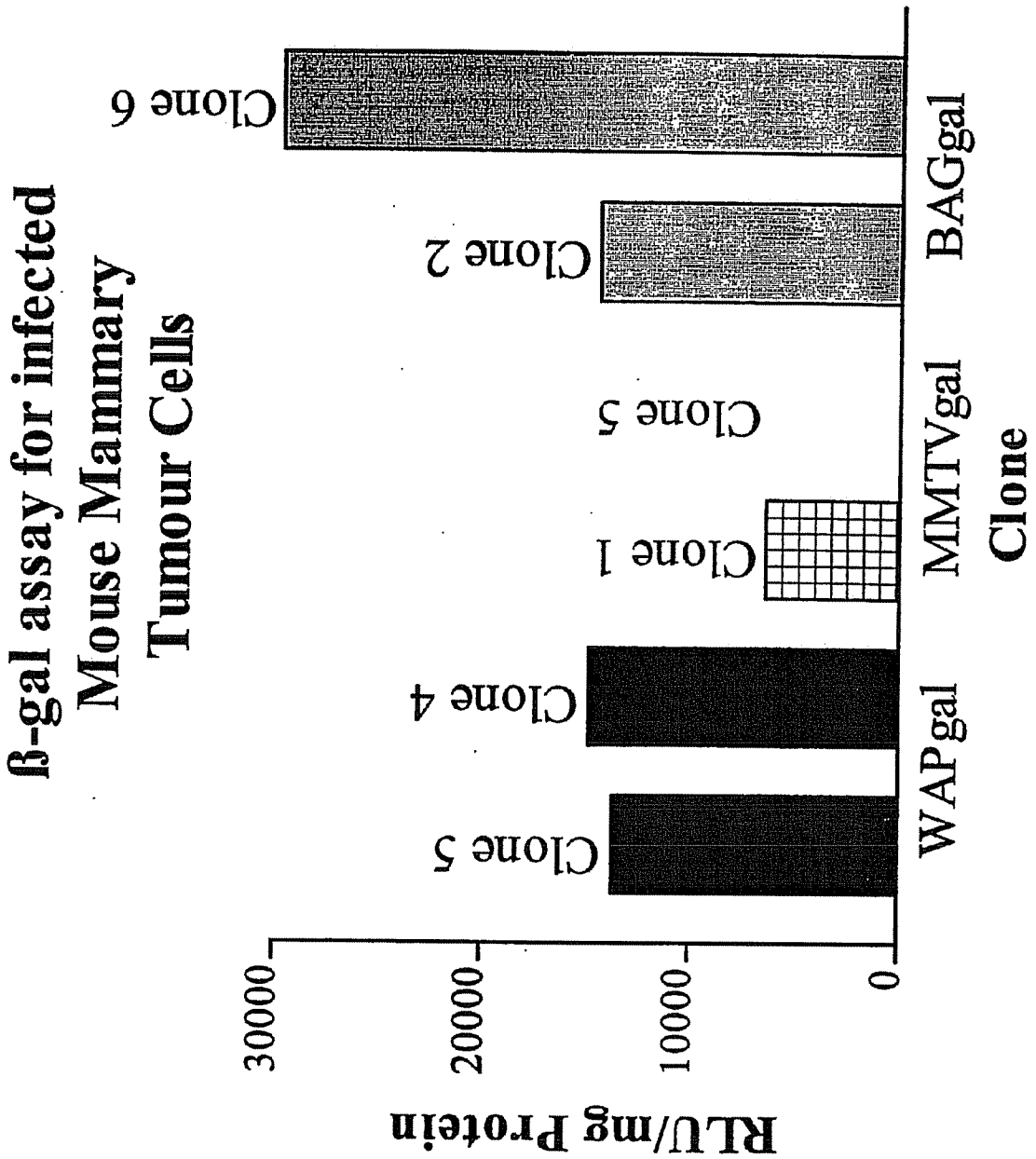
ß-gal assay for infected Mouse Mammary Tumour Cells
RLU/mg Protein
30000
20000
10000
0
Clone 5
Clone 4
Clone 1
Clone 5
Clone 2
Clone 6
WAPgal
MMTVgal
BAGgal
Clone

Fig. 10